The contribution of temperature, exposure intensity and visible light to the inhibitory effect of irradiation on acute chlamydial infection

Marti, Hanna ; Blenn, Christian ; Borel, Nicole

DOI: https://doi.org/10.1016/j.jphotobiol.2015.10.012

The contribution of temperature, exposure intensity and visible light to the inhibitory effect of irradiation on acute chlamydial infection

Hanna Marti[1], Christian Blenn[1], Nicole Borel[1]#

[1] Institute of Veterinary Pathology, University of Zurich, Vetsuisse Faculty, Winterthurerstrasse 268, 8057 Zurich, Switzerland

Email addresses: hanna.marti@uzh.ch, christian.blenn@mgb.ch, n.borel@access.uzh.ch

#Corresponding Author:

Nicole Borel, DVM, FVH, Dipl. ECVP

Institute of Veterinary Pathology, Vetsuisse Faculty

University of Zurich, Winterthurerstrasse 268

CH-8057 Zurich, Switzerland

Tel.: +41-44-635-8563

Fax.: +41-44-635-8934

Email: n.borel@access.uzh.ch

Abstract

Water-filtered infrared A (wIRA) is radiation with a spectrum ranging from 780 to 1400 nm. *Chlamydiaceae* are obligate intracellular bacteria associated with various diseases in both animals and humans. A recent *in vitro* study demonstrated that wIRA combined with visible light (wIRA/VIS) has potential as a non-chemical method for the treatment of chlamydial infections without adversely affecting the cell viability. The aim of this study was to investigate the influence of various factors on the effect of wIRA/VIS on acute chlamydial infection, namely the impact of temperature, exposure intensity and infectious dose (multiplicity of infection) as well as the efficacy of the visible light component. We demonstrate that non-thermal effects contribute to the inhibition of acute chlamydial infection. Visible light enhances the inhibitory effect of wIRA on extracellular bacteria (elementary bodies or EBs). Moreover, the inhibitory effect of wIRA/VIS following treatment of EBs prior to infection correlated with increased irradiation intensity. The infectivity of mature chlamydial inclusions was significantly reduced upon wIRA/VIS exposure at all irradiation intensities investigated, suggesting the contribution of host cell factors to the anti-chlamydial effect of wIRA/VIS in the late stage of the developmental cycle. The effect of irradiation was not influenced by the infectious dose.

Keywords

Chlamydia, developmental cycle, infectivity, titration by sub-passage, water-filtered infrared A, wIRA

1. Introduction

Water-filtered infrared A (wIRA) is infrared radiation with a spectrum of 780-1400 nm resulting from the light produced by a halogen bulb passing through a water cuvette to exclude wavelengths above 1400 nm and through a black filter to block visible light (VIS) [1]. Various clinical trials have shown that wIRA alone and in combination with visible light (wIRA/VIS) improves acute and chronic wound healing processes [2]. Moreover, two studies showed that wIRA/VIS treatment of abdominal wounds before or after surgery not only improved wound healing and oxygen partial pressure but also significantly reduced the rate of wound infections [3-4]. Surgical site infections are most often caused by the patient's endogenous flora. The common pathogens implicated in wound infections are the gram-positive *Staphylococcus aureus* and coagulase-negative staphylococci as well as the gram-negative *Escherichia coli* and *Enterococcus* ssp. [5]. Another study recently demonstrated that wIRA/VIS reduced the inclusion frequency of acute chlamydial infection without adversely affecting the cell viability or inducing cytotoxicity in host cells [6].

The *Chlamydiaceae* are a family of gram-negative bacteria consisting of one genus designated *Chlamydia (C.)* [7]. These obligate intracellular pathogens have a unique developmental cycle comprising an extracellular, infectious but metabolically less active stage (elementary bodies or EBs), which transforms into replicating reticulate bodies (RBs) before differentiating back into EBs [8]. They are responsible for a wide range of diseases, some of which are considered to be a public health concern in both human and veterinary medicine. For example, some strains of *C. trachomatis* are the causative agent of trachoma, the leading cause of infectious preventable blindness of human beings in developing countries, whereas other strains are recognized as the most important cause of bacterial sexually transmitted disease worldwide [9]. *C. pecorum*, a chlamydial species well-described in veterinary medicine, is associated with various medical conditions in pigs and ruminants including abortion, keratoconjunctivitis, pneumonia, polyarthritis and meningoencephalitis [10-11]. Furthermore, *C. pecorum*, together with other chlamydial species normally found in animals, such as *C. psittaci* and *C. suis*, has also been identified in the eye of trachoma patients in Nepal [12]. The treatment of choice for chlamydial infection is antimicrobial therapy; however, in general, the emergence of antibiotic-resistant bacteria raises concerns about the continued efficacy of this approach. Tetracycline-resistant *C. suis* strains, harboring the tetC resistance gene, have been isolated from asymptomatic pigs in the USA [13-14]. Horizontal transfer of this gene to various *Chlamydia* ssp. strains including *C.*

trachomatis has been demonstrated *in vitro* [15]. Furthermore, β-lactam antibiotics have often been reported to induce chlamydial persistence *in vitro* and *in vivo* [16]. Persistence is a reversible, viable but non-infectious chlamydial state. Though the importance of persistence *in vivo* is largely unknown, it might negatively impact both detection and successful antimicrobial treatment of chlamydial infection [17-18]. Taken together, new therapeutic strategies are needed to overcome current and developing problems with conventional treatment of chlamydial infections.

The aim of this study was to evaluate the impact of increasing irradiation intensity on wIRA/VIS-exposed chlamydial EBs and mature inclusions over a range of infectious doses, to assess the influence of visible light, and to evaluate the effect of irradiation in a temperature-controlled setting using both human (*C. trachomatis* serovar E and HeLa cells) and animal (*C. pecorum* 1710S and Vero cells) infection models.

2. Materials and Methods

2.1. Host cells and media

Vero 76 cells (African green monkey kidney cells, CRL 1587 American Type Culture Collection (ATCC), Manassas, VA, USA) and HeLa cells (Homo sapiens cervix adenocarcinoma, CCL-2 ATCC) were cultured at 37°C with 5% CO_2 in growth culture medium for cell propagation. Vero growth medium consisted of Minimal Essential Medium (MEM) with Earle's salts, 25 mM HEPES, without L-Glutamine (GIBCO, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (FCS, BioConcept, Allschwil, Switzerland), 4 mM GlutaMAX-I (200mM, GIBCO) and 0.2 mg/ml gentamycin (50 mg/ml, GIBCO). HeLa cell culture media were further supplemented with 1% MEM Non-Essential Amino Acids (MEM NEAA, 100x, GIBCO). Medium used for cell propagation intended for infection experiments was without gentamycin. Cells were grown on round glass coverslips (13 mm diameter, Sterilin Limited (Thermo Fisher Scientific), Cambridge, UK) in 24-well plates (Techno Plastic Products AG (TPP), Trasadingen, Switzerland) in 1 mL medium for infection experiments as previously described [6]. HeLa cells were seeded at a density of 3 x 10^5/well, Vero cells at a density of 2 x 10^5/well and infection experiments were performed when cells reached at least 90% confluency.

2.2. Chlamydial strains

In this study, two different strains of *Chlamydiaceae* were used for *in vitro* infection experiments: *Chlamydia (C.) pecorum* 1710S (isolate from a swine abortion, kindly provided by Prof. J. Storz, Baton

Rouge, LA, USA) and *C. trachomatis* serovar E (kindly provided by Prof. R. V. Schoborg, Johnson City, TN, USA). The isolate of the *C. trachomatis* strain was originally obtained from S. P. Wang and C.-C. Kuo (University of Washington, Seattle, WA, USA). The isolates were both propagated in HeLa cells. In summary, isolates were suspended in infection medium and used to infect HeLa cells. After centrifugation (1000g, 60 min), infection medium was replaced by growth medium plus gentamicin (0.2 mg/ml, GIBCO) and 1 ug/ml cycloheximide (Sigma-Aldrich, Inc., St. Louis, MO, USA). After an incubation period of 46 h (*C. trachomatis*) or 39 h (*C. pecorum*), monolayers were scraped and processed using sonication and centrifugation (500g, 10 min to remove cell debris and 10,000g, 45 minutes to pellet EBs). EBs were suspended in SPG medium and stored at -80 °C. SPG medium consisted of 218 mM sucrose (Sigma-Aldrich, Inc., St. Louis, MO, USA), 3.76 mM KH2PO4 (Sigma-Aldrich), 7.1 mM K2HPO4 (Merck Eurolab AG, Dietlikon, Switzerland) and 5 nm GlutaMAX-100 (GIBCO).

2.3. Irradiation

Cultures were either exposed to water-filtered infrared A in combination with visible light (wIRA/VIS) or wIRA alone for 20 min using a wIRA radiator (hydrosun 750, Hydrosun GmbH, Müllheim, Germany). The exposure intensity of wIRA/VIS and wIRA alone, respectively, depended on the experiment. The primary radiation spectrum of wIRA/VIS encompasses 400-1400 nm with negligible doses of UVA (400-315 nm; 25-30 W/m^2), UVB (315-280 nm; 15-25$\times 10^{-5}$ W/m^2) and UVC (280-100 nm; 5-21$\times 10^{-5}$ W/m^2 within the range of 250-280 nm) (measurements performed by the manufacturer). wIRA irradiation was attained by the use of a black filter (RG780, Hydrosun GmbH) resulting in a spectrum of 780 up to 1400 nm. The 24-well plates were placed in a thermostat-controlled water bath (SC100, Thermo Fisher Scientific, Newington, CT, USA), which maintained a temperature of 37 °C and was used as a cooling system for the irradiated cultures as previously described [19]. Non-irradiated controls were localized on the same plate and were kept at a suitable distance from the irradiated wells in order to avoid any direct and/or indirect irradiation influence (data not shown). Additionally, the temperature was monitored using a Voltcraft thermometer (Type 2ABAc, Philips, Kassel, Germany) as previously described [6].

2.4. Study design

2.4.1. Infection Experiments

The experiments were organized in two different treatment groups: i) *Chlamydia*-infected cells with irradiation and ii) *Chlamydia*-infected cells without irradiation. Non-infected and non-irradiated cells were included as negative controls (data not shown). Vero and HeLa cells were infected with either *C. pecorum* or *C. trachomatis* at a multiplicity of infection (MOI) of 1, 0.1 or 0.01 in 1 mL infection medium (growth culture medium without FCS and gentamycin). After centrifugation for 1 hour (h) at 1000 g and 25 °C, infection medium was replaced by incubation medium (growth culture medium without gentamycin) containing 1 ug/ml cycloheximide (Sigma-Aldrich) and cultures were incubated at 37 °C as previously described [20].

2.4.2. Irradiation of chlamydial EBs prior to host cell infection

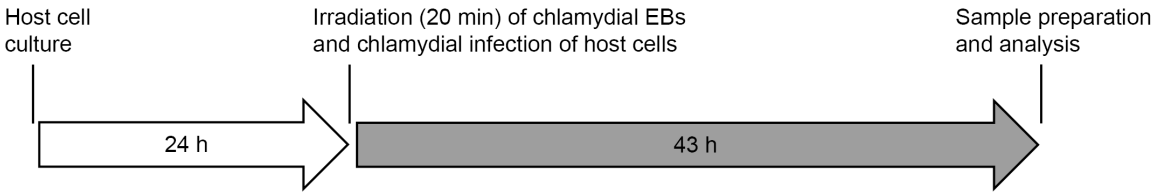
Chlamydial EBs at a MOI 0.01, 0.1 or 1 were irradiated in 1 mL infection medium for 20 min before infection of host cells. Non-irradiated EBs were used as controls. After an incubation period of 43 h, cultures were subjected to i) immunofluorescence microscopy, or ii) infectious titer analysis, as appropriate (Figure 1A). *C. pecorum* EBs were used to infect Vero and HeLa monolayers. *C. trachomatis* EBs were applied to HeLa cells only.

2.4.3. Single-dose irradiation 40 hours post infection

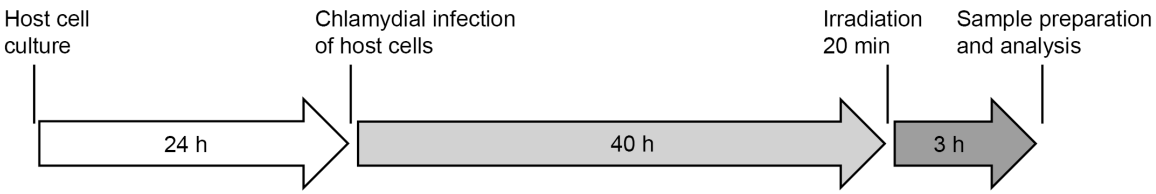
For single-dose irradiation, HeLa and Vero monolayers were infected with *C. trachomatis* or *C. pecorum*. After an incubation period of 40 h at 37 °C, cultured cells were irradiated for 20 min. Cultures were incubated for another 3 h at 37 °C under standard culture conditions before being subjected to i) immunofluorescence microscopy, or ii) infectious titer analysis, as appropriate (Figure 1B). Vero monolayers were infected with *C. pecorum* and HeLa cells were infected with *C. trachomatis (*Figure 1C*)*.

Figure 1.

A)



B)



C)

Host cell	*Chlamydia* species
Vero	*Chlamydia pecorum*
HeLa	*Chlamydia pecorum*
	Chlamydia trachomatis

Figure 1. Study Design. (2 columns)

A) Diagram of the irradiation prior to infection procedure. EBs were irradiated for 20 min and used to infect host cells. After incubating infected cell cultures for 43 h, samples were further processed and analyzed. B) Diagram of the infection and single-dose irradiation procedure. Host cells were infected, incubated for 40 h and irradiated for 20 min. After another incubation period of 3 h, samples were further processed and analyzed. C) Table shows host cells and chlamydial strains investigated within this study.

2.5. Immunofluorescence microscopy

Monolayers were fixed with absolute methanol (-20 °C) for 10 min and immunolabeled as described [20]. Briefly, chlamydial inclusions were detected using a *Chlamydiaceae* family-specific mouse monoclonal antibody directed against the chlamydial lipopolysaccharide (LPS, Clone ACI-P, 1:200; Progen, Heidelberg, Germany) and a 1:500 diluted Alexa Fluor 488-conjugated secondary goat anti-mouse antibody (Molecular Probes, Eugene, OR, USA). Host and chlamydial DNA were labeled using 1:1000 diluted 4', 6-Diamidin-2'-phenylindoldihydrochlorid (DAPI, 1 μg/mL, Molecular Probes). Coverslips were mounted with FluoreGuard Mounting (Hard Set, ScyTek Laboratories Inc., Logan, UT,

USA) on glass slides and assessed using a Leica DMLB fluorescence microscope (Leica Microsystems, Wetzlar, Germany) under oil immersion at 1000-fold magnification with a 100x objective (PL FLUOTAR 100x/1.30, OIL, ∞/0.17/D, Leica Microsystems) and a 10x ocular objective (Leica L-Plan 10x/25 M, Leica Microsystems). In total, 200 cell nuclei and all chlamydial inclusions per examined field were counted as previously described [6]. Microscopic images were captured with the BonTec measuring and archiving software (BonTec, Bonn, Germany) using a UI-2250SE-C-HQ camera (uEye, IDS Imaging Development Systems GmbH, Obersulm, Germany).

2.6. Chlamydial titration by sub-passage

Monolayers were scraped together with supernatant at 43 hpi and stored at -80°C, followed by sub-passage on the respective host cell as shown previously [6]. Fixation and immunofluorescence staining was performed as described in subsection 2.5. The number of inclusions in 30 random microscopic fields per sample was determined using a Leica fluorescence microscope at 200-fold magnification with a 20x objective (PL FLUOTAR 20x/0.50 PH2, ∞/0.17/B) and a 10x ocular objective (Leica L-Plan 10x/25 M, Leica Microsystems). The number of inclusion-forming units (IFU) in undiluted inoculum was then calculated and expressed as IFU per ml inoculum according to previously published methods [21].

2.7. Statistical analysis

All statistical analyses were performed with the GraphPad Quickcalcs software (www.graphpad.com/quickcalcs). If not stated differently, all results are displayed as means ± standard deviation (SD) of the indicated number of experiments. The significance of differences was estimated by *t* test, and a p-value of < 0.05 was considered significant.

3. Results

3.1. Non-thermal effects contribute to the reducing effect of wIRA/VIS exposure

In a recent study, we demonstrated that thermal effects were responsible, at least in part, for the reduced chlamydial activity subsequent to wIRA/VIS irradiation [6]. Briefly, we mimicked the temperature raise induced by 20 min of wIRA/VIS at 3700 W/m^2 by increasing the water bath temperature to 41 °C. Comparing these treatments by exposing *Chlamydia*-infected cells either to

wIRA/VIS irradiation or water bath treatment for 20 min at 40 hours post infection (hpi), we demonstrated that an increase in water bath temperature alone reduced the number of chlamydial inclusions per nucleus. In the current study, we eliminated wIRA/VIS-induced thermal effects by lowering the water bath temperature to 34 °C. The resulting intra-well temperature on top of the coverslip (at the bottom of the well) after the 20 min irradiation period (35.93±0.38 °C) was identical to non-irradiated control wells (36.10±0.1 °C) at a water bath temperature of 37 °C (Figure 2A, Figure S1A). *C. trachomatis*-infected HeLa cells (MOI 1) were exposed to wIRA/VIS at 3700 W/m^2 for 20 min 40 hpi at a water bath temperature of 34 °C and 37 °C, respectively. Non-irradiated controls were included for both conditions. Following irradiation, cultures were incubated at 37 °C for three hours before they were collected for sub-passage titer analysis. IFU/mL was calculated and expressed as percentage of their respective control. The reduction in the number of IFU/ml following irradiation in a temperature-controlled setting (water bath 34 °C, 65.99% ± 15.97%) was similar compared to irradiation at a water bath temperature of 37 °C (65.75% ± 16.29%, Figure 2B). There was no significant difference between the controls (data not shown).

Figure 2.

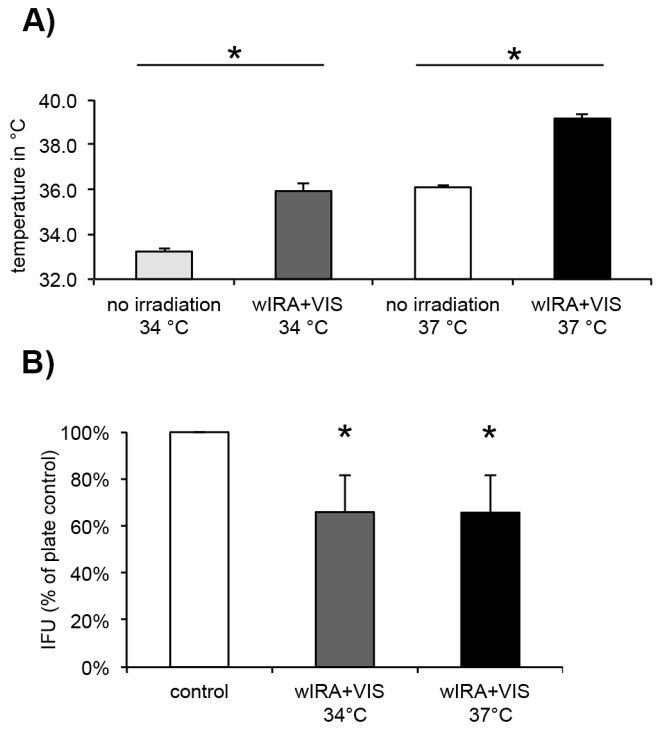


Figure 2. Non-thermal effects contribute to the anti-chlamydial effect of wIRA/VIS exposure. (1 column)

A) 1 mL of cell culture medium was added per well and irradiated with wIRA/VIS (3700 W/m^2) for 20 min at a water bath temperature of 34 °C (wIRA+VIS, 34 °C) and 37 °C (wIRA+VIS, 37 °C), respectively. Non-irradiated medium was used as controls (no irradiation). Shown is the mean intra-well temperature of three wells per condition after 20 min of irradiation (mean ± SD; * $p < 0.05$; *t* test). B) *C. trachomatis*-infected HeLa cells (MOI 1) were irradiated 40 hours post infection (hpi) with 3700 W/m^2 at a water bath temperature of 34 °C and 37 °C, respectively. Cultures were further incubated at 37 °C, collected 43 hpi and subjected to sub-passage titer analysis. Inclusion forming units per mL (IFU/mL) are shown as percent of control (mean ± SD; * $p < 0.05$; $n \geq 3$; *t* test).

3.2. Visible light contributes to the increase of intra-well temperature at high exposure intensities

Next, we investigated possible differences in the anti-chlamydial effect of wIRA/VIS and wIRA alone both at low and high exposure intensities. First, using a water bath set to 37 °C, we measured the intra-well temperature during an irradiation period of 20 min by exposing cell culture medium to wIRA/VIS (wIRA+VIS 1000, wIRA+VIS 3700) at the applied dose of 1000 W/m^2 (Figure 3A) and 3700 W/m^2 (Figure 3B), respectively. The corresponding exposure intensity of wIRA alone (wIRA 650, wIRA 2000) was 650 and 2000 W/m^2, respectively. There was no difference in intra-well temperature between wIRA/VIS (36.73 °C ± 0.06 °C) and wIRA alone (36.73 °C ± 0.12 °C) at a low irradiation dose after 20 min of irradiation. Furthermore, the resulting intra-well temperature for both conditions did not exceed 37 °C, but the difference compared to the control (36.27 °C ± 0.06 °C) was significant. However, at high exposure intensities, intra-well temperature following wIRA/VIS treatment (39.17 °C ± 0.15 °C) was significantly increased compared to irradiation with wIRA alone (38.03 °C ± 0.15 °C).

Figure 3.

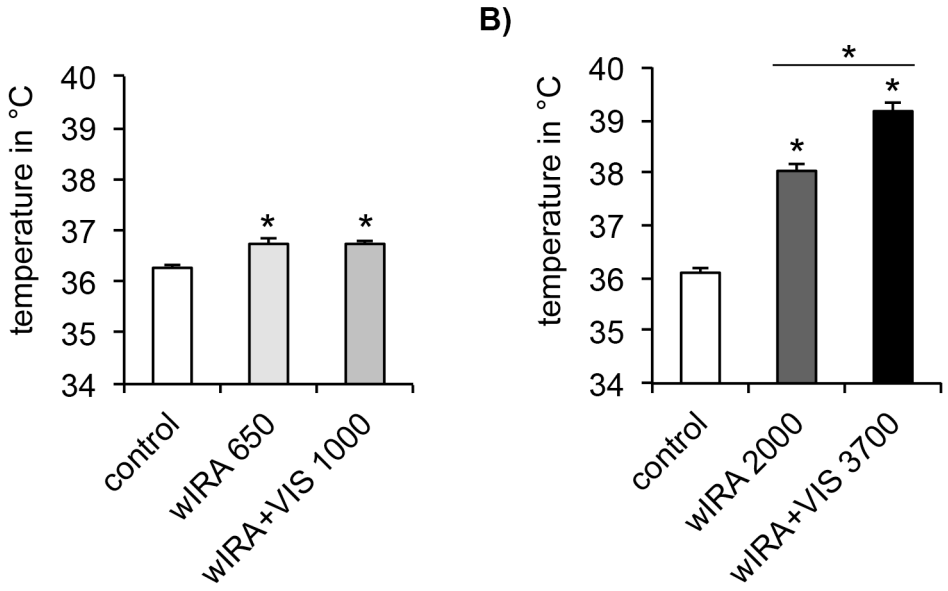


Figure 3. Visible light contributes to the increase of intra-well temperature at high exposure intensities. (1.5 columns)

1 mL of cell culture medium was added per well and irradiated with wIRA/VIS (wIRA+VIS) or wIRA alone (wIRA) for 20 min at A) 1000 and B) 3700 W/m^2, respectively. The corresponding exposure intensity of wIRA alone was A) 650 and B) 2000 W/m^2 (wIRA 650, 2000), respectively. The water bath temperature was 37 °C. Non-irradiated medium was used as control. Shown is the mean intra-well temperature of three wells per condition after 20 min of irradiation (mean ± SD; * n=3, $p < 0.05$; *t* test).

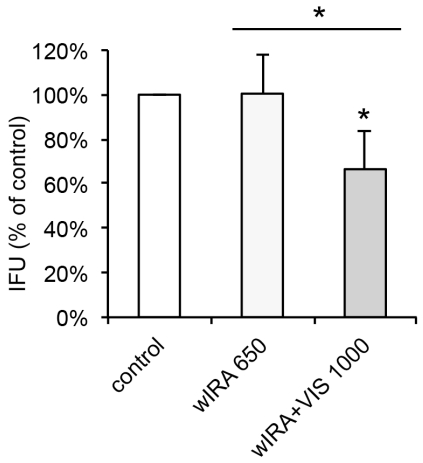
3.3. The reduction in infectivity of mature chlamydial inclusions by wIRA alone is independent of the addition of visible light

To determine the possible contribution of visible light to the effect of wIRA/VIS irradiation on mature chlamydial inclusions, we used both human (*C. trachomatis*-infected HeLa cells) and animal (*C. pecorum*-infected Vero cells) cell culture models as previously described [6]. Cells were infected with MOI 0.1 and incubated for 40 hours prior to irradiation for 20 min with either wIRA/VIS or wIRA alone at a water bath temperature of 37 °C. The exposure intensity of wIRA/VIS was 1000 W/m^2 (wIRA+VIS 1000) at low and 3700 W/m^2 (wIRA+VIS 3700) at high irradiation doses. The corresponding exposure intensities with wIRA alone were 650 W/m^2 (wIRA 650) and 2000 W/cm^2 (wIRA 2000), respectively. Non-irradiated, infected HeLa or Vero cultures were used as controls. After a further incubation period

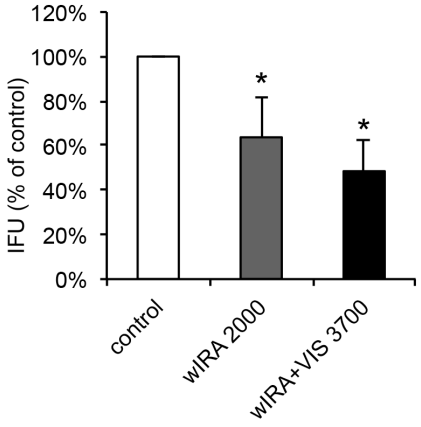
of three hours, cultures were collected for sub-passage titer analysis. Following wIRA/VIS irradiation at low exposure intensity, the IFU/mL was reduced compared to the control for both *C. trachomatis* in HeLa (66.53% ± 17.18%, Figure 4A) and for *C. pecorum* in Vero cells (50.40% ± 18.06%). However, only for *C. pecorum* in Vero cells was IFU/ml significantly reduced by irradiation with wIRA alone (63.98% ± 6.18%, Figure 4C). In contrast, there was no significant difference between the reduction caused by wIRA/VIS treatment and wIRA alone, regardless of the cell culture model, at high irradiation doses. For *C. pecorum*, IFU/mL was 26.34% ± 10.71% for wIRA/VIS and 50.58% ± 13.81% for wIRA alone compared to the control (Figure 4B), whereas for *C. trachomatis*, IFU/ml was 48.49% ± 14.14% and 63.72% ± 17.82%, respectively (Figure 4D). Similar results were obtained for *C. trachomatis* at MOI 1 (Figure S2A-B) and MOI 0.01 (Figure S2C-D).

Figure 4.

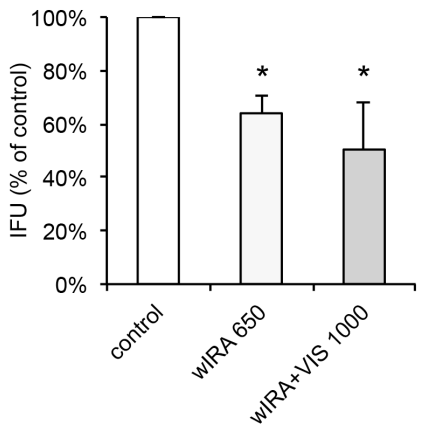
A) *C. trachomatis* in HeLa



B) *C. trachomatis* in HeLa



C) *C. pecorum* in Vero



D) *C. pecorum* in Vero

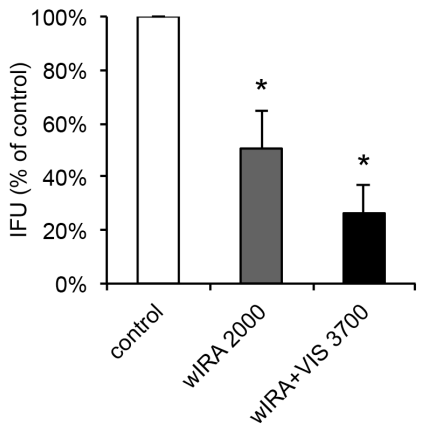


Figure 4. The reduction in infectivity of mature chlamydial inclusions by wIRA alone is independent of the addition of visible light. (2 columns)

HeLa cells infected with *C. trachomatis* (MOI 0.1) were irradiated or not with A) wIRA/VIS (wIRA+VIS 1000) or wIRA alone (wIRA 650), and B) wIRA+VIS 3700 or wIRA alone (wIRA 2000) at 40 hours post infection (hpi) for 20 min (water bath temperature 37 °C), respectively. Non-irradiated cultures were used as controls. Cultures were incubated for another three hours and then collected for sub-passage titer analysis. Inclusion forming units per mL (IFU/mL) are shown as percent of control (mean ± SD; * $p < 0.05$; $n \geq 3$; *t* test).

Vero cells were infected with *C. pecorum* at MOI 0.1 and irradiated or not at 40 hours post infection (hpi) for 20 min (water bath temperature 37 °C) with C) wIRA+VIS 1000 (1000 W/m^2) or wIRA alone (wIRA 650) and D) wIRA+VIS 3700 (3700 W/m^2) or wIRA alone (wIRA 2000), respectively. Non-irradiated cultures were used as controls. Cultures were incubated for another three hours and then collected for sub-passage titer analysis. Inclusion forming units per mL (IFU/mL) are shown as percent of control (mean ± SD; * $p < 0.05$; $n \geq 3$; *t* test).

3.4. The inhibitory effect of wIRA/VIS on the infectivity of EBs is influenced by visible light

In a previous publication, we reported that irradiation of EBs of two different chlamydial strains with wIRA/VIS at 3700 W/m^2 for twenty minutes prior to infection of their respective host cells dramatically reduced the infectivity of EBs [6]. In the current study, it was our goal to determine the effect of visible light on extracellular EBs at both low (1000 W/m^2) and high (3700 W/m^2) irradiation doses using irradiated *C. trachomatis* and *C. pecorum* EBs to subsequently infect HeLa cells or HeLa and Vero cells, respectively. After 43 h of incubation, cultures were either fixed in methanol, immunolabeled and analyzed or collected for subsequent titration by sub-passage as described in the Materials and Methods section (“2.6. Chlamydial titration by sub-passage”). The infectivity rate of each condition 43 hpi was evaluated (data not shown). Representative microscopic pictures were taken at 1000-fold magnification (Figure 5). While the infectivity of EBs was reduced for both *C. pecorum* in Vero (70.88% ± 16.26%, Figure 6A) and *C. trachomatis* in HeLa cells (72.83% ± 8.61%, Figure 6C) following wIRA/VIS treatment of extracellular EBs at a low exposure intensity, wIRA alone only significantly reduced the IFU/mL of *C. pecorum* in Vero cells (82.83% ± 5.97%, Figure 6A). As opposed to this, the infectivity of EBs was reduced for both *C. pecorum* in Vero and *C. trachomatis* in HeLa cells using wIRA alone at high irradiation doses. However, there was a marked difference

between wIRA/VIS and wIRA without visible light for both cell culture models. Sub-passage titer analysis confirmed that IFU/mL was reduced more efficiently by wIRA/VIS treatment of *C. pecorum* EBs before infecting Vero cells (23.17% ± 6.37%) compared to wIRA alone (67.41% ± 12.92%, Figure 6B). Irradiation of *C. trachomatis* EBs prior to infection of HeLa cells provided similar results with 10.85% ± 5.42% for wIRA/VIS and 79.81% ± 7.27% for wIRA without visible light, respectively (Figure 6D). For *C. pecorum* in HeLa cells, the infectivity of EBs was reduced following wIRA/VIS treatment at both low (67.23% ± 9.82%) and high (33.77% ± 4.82%) irradiation doses, but not if EBs were irradiated with wIRA alone (Figure S3).

Figure 5.

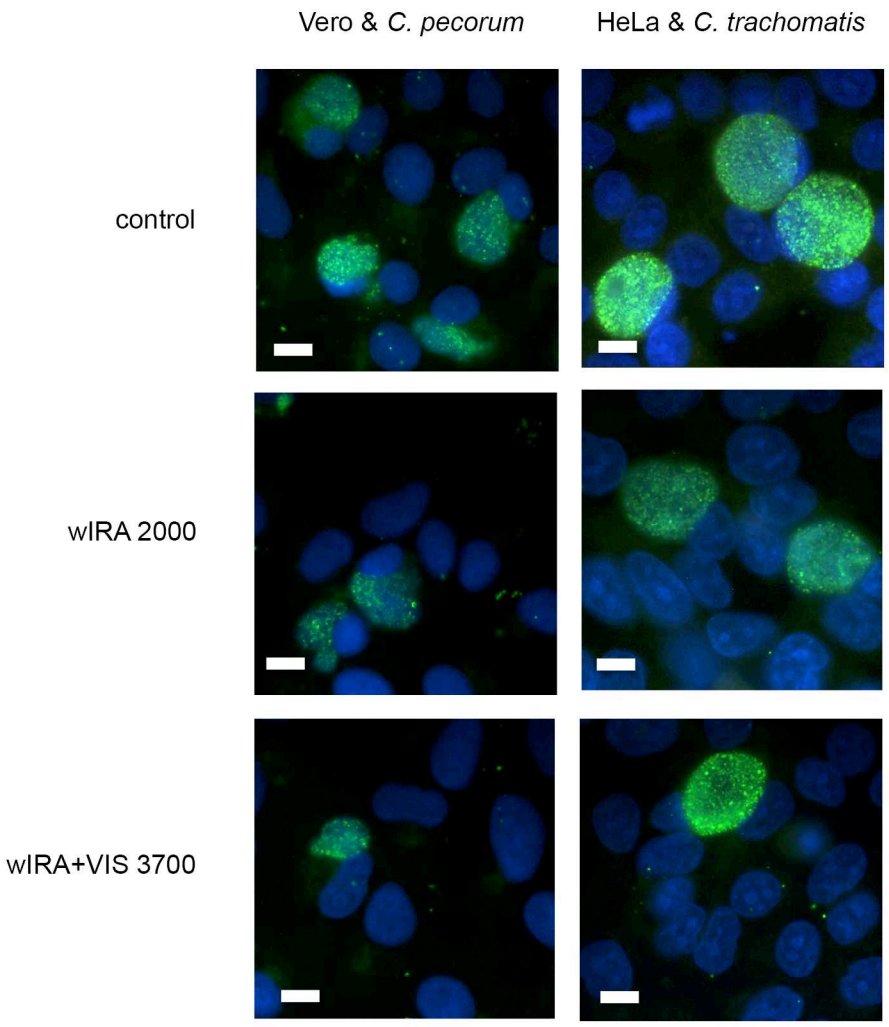


Figure 5. Immunofluorescence Microscopy Images. (1.5 columns)

Chlamydial EBs (*C. pecorum, C. trachomatis*) at MOI 0.1 were exposed to wIRA/VIS (wIRA+VIS 3700, exposure intensity: 3700 W/m^2) or wIRA alone (wIRA 2000) prior to infection of HeLa and Vero cells. Cultures were fixed 43 hours post infection (hpi) and immunolabeled with anti-chlamydial LPS (green) and DAPI (blue). Representative microscopic pictures at 1000x magnification are shown. White scale bars = 10 µm.

Figure 6.

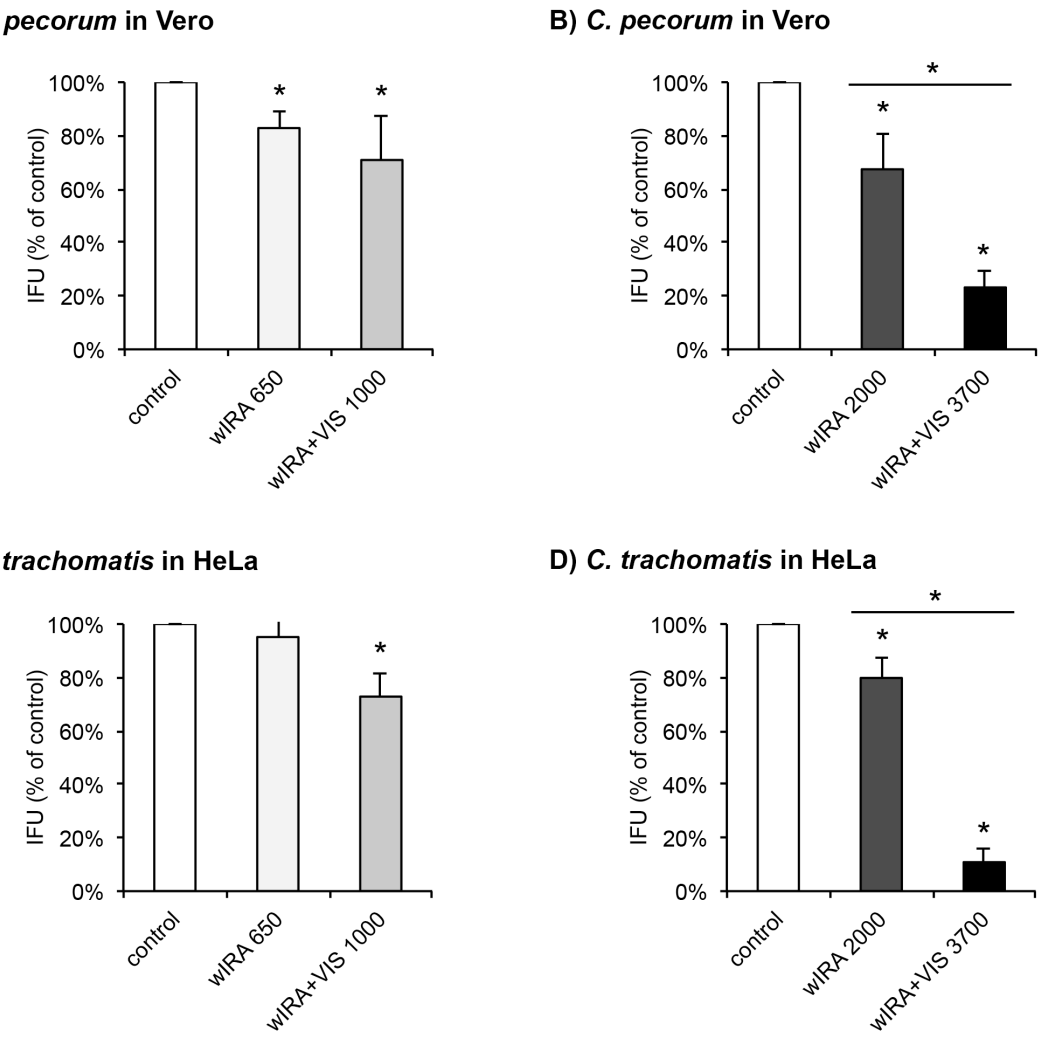


Figure 6. The inhibitory effect of wIRA/VIS on the infectivity of EBs is influenced by visible light. (2 columns)

A) EBs of *C. pecorum* were irradiated with wIRA/VIS (wIRA+VIS 1000, 1000 W/m^2) or wIRA alone (wIRA 650, 650 mW/cm^2) and B) wIRA+VIS 3700 (3700 mW/cm^2) or wIRA alone (wIRA 2000, 2000 mW/cm^2), respectively, prior to infection of Vero cells at a MOI of 0.1 (water bath 37 °C). Cultures

were incubated for 43 hours and collected for sub-passage titer analysis. Non-irradiated EBs were used as controls. Inclusion forming units per mL (IFU/mL) are shown as percent of control (mean ± SD; * p = 0.05; n = 3; *t* test). C) *C. trachomatis* EBs were irradiated with wIRA/VIS (wIRA+VIS 1000) or wIRA alone (wIRA 650) and D) wIRA+VIS 3700 or wIRA 2000, respectively, prior to infection of HeLa cells at a MOI of 0.1 (water bath 37 °C). The numbers of inclusion forming units per mL (IFU/mL) are represented as percent of control (mean ± SD; * p = 0.05; n = 3; *t* test).

3.5. The inhibitory effect of wIRA/VIS on the infectivity of EBs depends on irradiation intensity but is independent of the chlamydial infectious dose

To further evaluate the influence of the chlamydial infectious dose and wIRA/VIS intensity on the anti-chlamydial effect of irradiation, *C. trachomatis* EBs at MOI 0.01, 0.1 and 1 were irradiated with wIRA/VIS at low (1000 W/m^2, wIRA+VIS 1000), intermediate (2000 W/m^2, wIRA+VIS 2000) and high (3700 W/m^2, wIRA+VIS 3700) exposure intensities for 20 min at a water bath temperature of 37 °C prior to infection of HeLa cells. After 43 hours of incubation, cultures were collected and subjected to titration by sub-passage. IFU/mL was calculated and expressed as percentage of the control. Regardless of the MOI, wIRA/VIS reduced the infectivity of EBs in a dose-dependent manner. At MOI 1, the IFU/mL for wIRA+VIS 3700, wIRA+VIS 2000 and wIRA+VIS 1000 was 30.75% ± 11.89%, 61.16% ± 15.78% and 81.87% ± 12.70%, respectively (Figure 7A), whereas at MOI 0.1, it was 10.85% ± 5.42%, 45.21% ± 8.08% and 72.83 ± 8.61%, respectively (Figure 7B). Finally, MOI 0.01 yielded 13.30% ± 3.26% subsequent to treatment with wIRA+VIS 3700, 36.46% ± 2.94% with wIRA+VIS 2000 and 90.25% ± 35.81% with wIRA+VIS 1000, respectively (Figure 7C). At low irradiation doses, the reduced infectivity was significant only at MOI 0.1. Prior to the infection experiments, temperature profiles as shown in Figures 2A, 3 and S1A were established, indicating that, at all irradiation intensities evaluated, the addition of VIS to wIRA irradiation did not affect the temperature measured in the wells (Figure S1B).

Figure 7. *C. trachomatis* in HeLa

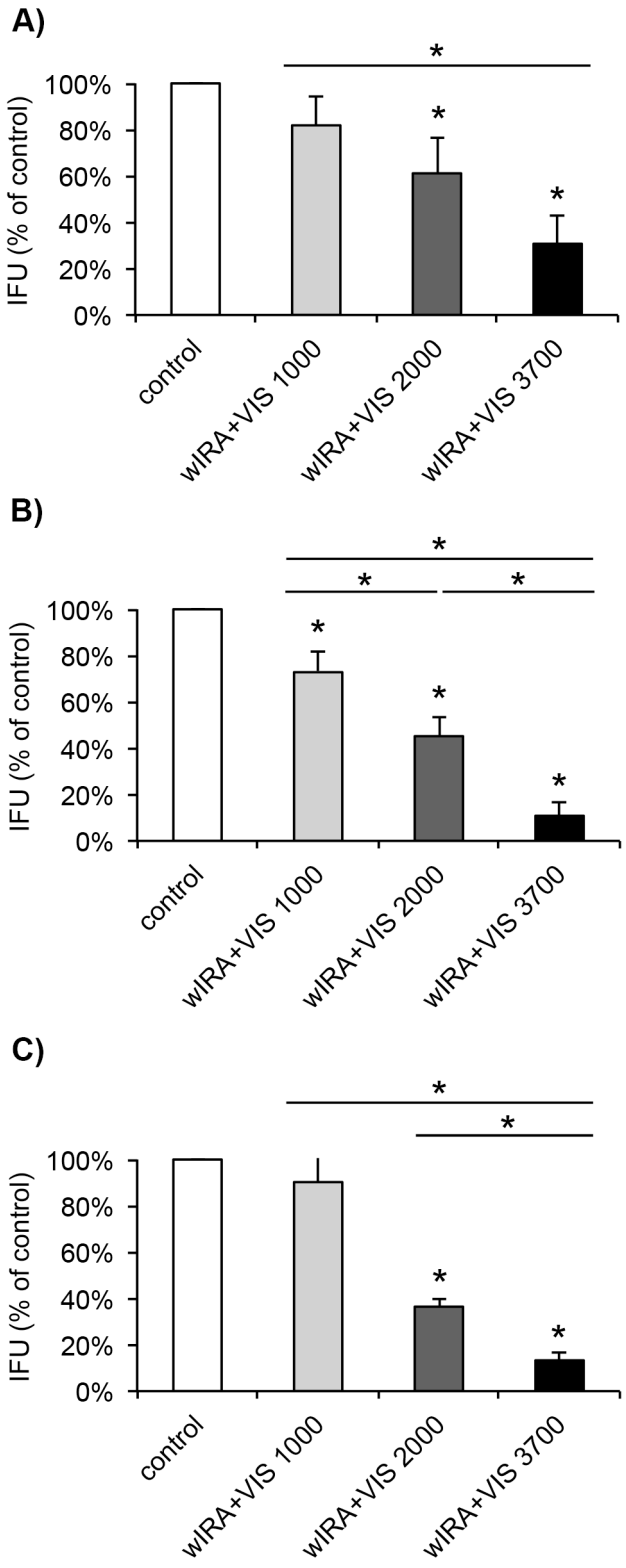


Figure 7. The inhibitory effect of wIRA/VIS on the infectivity of EBs depends on the irradiation intensity but is independent of the infectious dose. (1 column)

C. trachomatis EBs were exposed to wIRA/VIS at various irradiation intensities (1000, 2000, 3700 W/m^2) prior to infection of HeLa cells at a A) MOI of 1, B) MOI of 0.1 and C) MOI of 0.01 (water bath 37 °C). Non-irradiated EBs were used as controls. After 43 hours of incubation, cultures were collected and subjected to sub-passage titer analysis. Inclusion forming units per mL (IFU/mL) are presented as percent of control (mean ± SD; * $p < 0.05$; $n = 3$; *t* test).

3.6. The reduction in infectivity of mature chlamydial inclusions caused by wIRA/VIS is independent of both irradiation intensity and chlamydial infectious dose

To complete our evaluation of the influence of wIRA/VIS at different exposure intensities on *Chlamydia* at various MOI, we used the same setup as described in subsection 3.5, but, instead of irradiating EBs prior infection, we irradiated *C. trachomatis*-infected HeLa cells 40 hpi. Cultures were collected and further processed for sub-passage titer analysis three hours after wIRA/VIS treatment (43 hpi). Again, the result was not influenced by the MOI. Additionally, we observed that wIRA/VIS similarly reduced IFU/ml after treatment of mature chlamydial inclusions, regardless of the irradiation intensity. At MOI 1, IFU/mL as percent of the control was 66.58% ± 7.08% for wIRA+VIS 3700, 75.46% ± 11.42% for wIRA+VIS 2000 and 63.69% ± 6.54% for wIRA+VIS 1000 (Figure 8A). Almost identical results were found at MOI 0.1 with 48.49% ± 14.14%, 56.59% ± 26.54% and 66.53% ± 17.18% Figure 8B, and at MOI 0.01 with 43.48% ± 20.52%, 62.97% ± 17.95% and 65.23% ± 20.57% for wIRA+VIS 3700, wIRA+VIS 2000 and wIRA+VIS 1000, respectively (Figure 8C).

Figure 8. *C. trachomatis* in HeLa

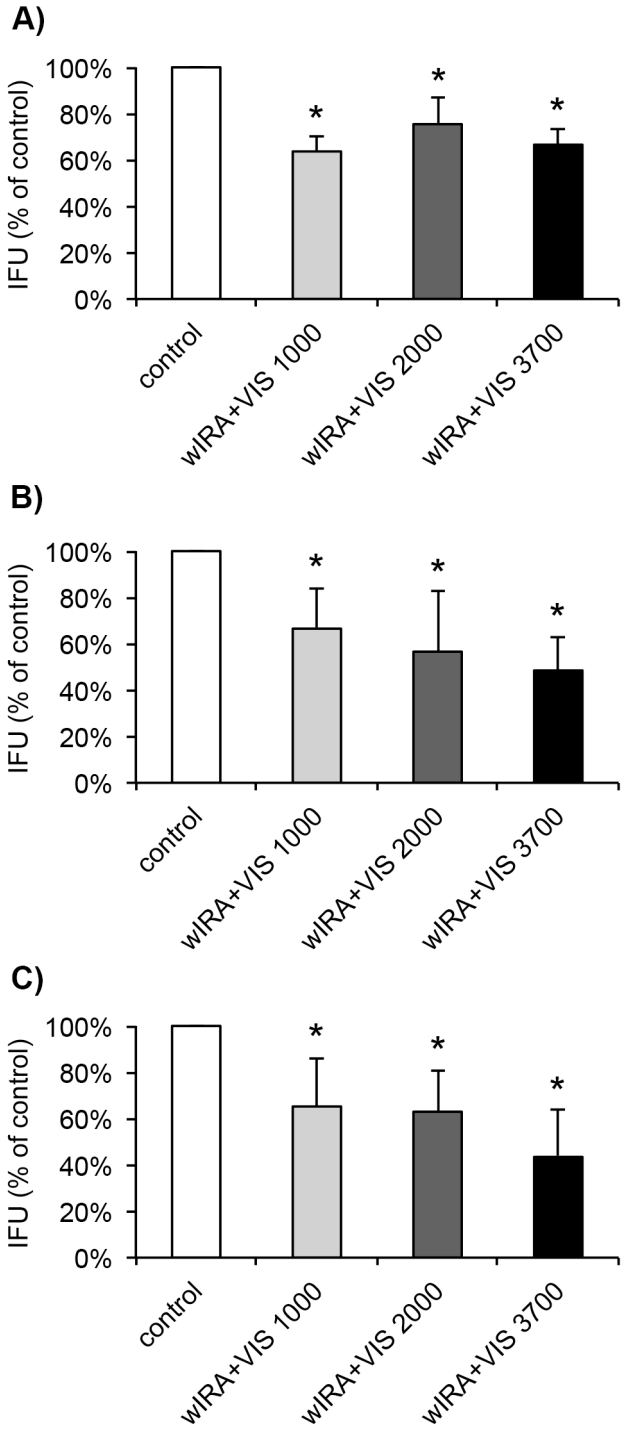


Figure 8. The reduction in infectivity of mature chlamydial inclusions caused by wIRA/VIS is independent of both irradiation intensity and infectious dose. (1 column)

HeLa cells infected with *C. trachomatis* at an A) MOI of 1, B) MOI of 0.1, and C) MOI of 0.01 were irradiated or not with wIRA/VIS at various intensities (1000, 2000, 3700 W/m^2) 40 hours post infection (water bath temperature 37 °C) and incubated for another three hours. Cultures were then collected

and sub-passage titer analysis was performed. The numbers of inclusion forming units per mL (IFU/mL) are shown as percent of control (mean ± SD; * $p < 0.05$; $n \geq 3$; *t* test).

4. Discussion

Blinding trachoma afflicts over 300 million people globally, most of whom live in developing countries [22]. Recommended treatment for trachoma includes either a single dose of azithromycin or topical application of tetracycline twice daily for six weeks [16, 22-24]. However, the recent detection of tetracycline-resistant *C. suis* strains in the USA and Europe stresses the importance of developing alternative treatment for chlamydial infection [13, 25-27]. This issue becomes even more urgent if we consider that mixed infections with *C. trachomatis* and *C. suis* were found in the eyes of trachoma patients in Nepal [12] and that horizontal transfer of the tetC resistance gene from the *C. suis* R19 strain to *C. trachomatis* was shown *in vitro* [15, 28].

In a recent publication, the first to our knowledge, to evaluate the effects of wIRA/VIS on Chlamydia *in vitro*, we demonstrated that water-filtered infrared A in combination with visible light (wIRA/VIS) has potential as an easily applied, non-chemical technique to inhibit acute chlamydial infection *in vitro* without inducing cytotoxicity in host cells. For example, we showed that wIRA/VIS did not activate the extracellular signal-regulated kinase 1/2 (ERK 1/2) [6]. ERK 1/2, in turn, directly regulates Matrix Metalloproteinase (MMP)-1, an enzyme involved in the process of premature skin aging and which is expressed in response to the potentially carcinogenic ultraviolet light (UV)B and UVA but also infrared A radiation [29-34]. Moreover, the MMPs serve as pro-fibrotic endopeptidases and are important factors in the scarring process of trachoma [35]. Furthermore, using the resorufin-based AlamarBlue assay, we showed that the viability of HeLa cells is not impaired after wIRA/VIS treatment for 4h at the highest possible dose of 3700 W/m^2 [6]. Also in the previous report, in order to gain first insights into the mechanism of action of the wIRA/VIS anti-chlamydial effect, instead of irradiating the cells with wIRA/VIS 40 hpi, we increased the water bath temperature thereby mimicking the irradiation-induced temperature rise within the wells from approximately 36 °C up to 39 °C. The reduction in the number of mature chlamydial inclusions (percent infection) compared to the control 43 hpi was similar to that caused by wIRA/VIS treatment. These findings led us to the hypothesis that the inhibiting effect of wIRA/VIS is likely caused, at least in part, by a thermal-induced mode of action [6].

In the current study, we continue to investigate the mechanism(s) of action of the wIRA/VIS anti-chlamydial effect. We demonstrate that the infectivity of mature chlamydial inclusions is also inhbited when the irradiation-induced increase of intra-well temperature is prevented by using a temperature-controlled setting for wIRA/VIS exposure. We show here that both thermal and non-thermal effects play a role in the anti-chlamydial mechanism of wIRA/VIS.

However, these components of irradiation are not significantly additive, which is in accordance with published experiments regarding solar disinfection (SODIS). SODIS is a non-chemical water treatment system used in developing countries, whereby light and thermal energy of the sun is used to purify microbially contaminated drinking water. A synergistic effect of light energy, mainly produced by UVB (280-320 nm) as well as UVA (320-400 nm), and thermal energy, induced by infrared irradiation, was only observed for water temperatures exceeding 45 °C [36-38].

Our results herein show that visible light enhances the inhibitory effect of wIRA on extracellular EBs at all exposure intensities investigated. Moreover, the infectivity of extracellular EBs was also significantly reduced by wIRA irradiation alone at high irradiation doses. These findings are supported by empiric data showing that chlamydiae have a remarkably high environmental survival rate, especially in cool, dry and dark conditions [39]. For example, EBs of *C. abortus*, the causing agent of enzootic ovine abortion, remain infectious in the environment for at least several days, and cold outdoor temperatures close to or below freezing may prolong their viability for weeks or even months [40-41]. These observations suggest that light and elevated temperatures reduce the viability and infectivity of extracelluar EBs, which corresponds to the results of our *in vitro* experiments.

In contrast to irradiation of EBs alone, we show here that the infectivity of mature chlamydial inclusions is equally reduced in both the presence and absence of visible light, indicating that visible light does not contribute to the anti-chlamydial effect on mature chlamydial inclusions. This suggests that the host cell itself, in response to wIRA/VIS exposure, plays a role in exerting the wIRA/VIS-dependent inhibitory effect on intracellular chlamydiae, possibly by eliciting a pro-inflammatory response.

This hypothesis is supported by other observations made in this study and our previous study. First, the anti-chlamydial efficacy of irradiation differed if the same treatment parameters were applied at the very beginning of the developmental cycle (extracellular EBs) or at a late stage of infection (40 hpi). Second, wIRA/VIS treatment at 40 hpi similarly reduced the number of RBs and EBs within an inclusion at 40 hpi [6], thus indicating that intracellular EBs and RBs are equally vulnerable to wIRA/VIS after a single dose of irradiation. Third, we detected an up-regulation of pro-inflammatory

cytokines (MIF/GIF, Serpin E1, RANTES, IL-6, IL-8) and chemokines (IL-16, IP-10, ENA-78, MIG, MIP-1α/β) at 43 hpi subsequent to wIRA/VIS exposure, which was identical to what we detected after either chlamydial infection alone or infection combined with wIRA/VIS treatment [6]. It is also possible that intrainclusional *Chlamydia* are protected from the extracellular environment (including visible light) similar to *Legionella pneumophila* within amoebae [42].

Unfortunately, the exact mechanism of the interaction between cellular structures and wIRA irradiation is still not entirely understood. While the formation of reactive oxgen specimen (ROS) and mitochondrial superoxide formation as well as changes in the mitochondrial membrane potential have been reported in connection with a wIRA irradiation dose of 1050 W/m^2 and unknown thermal fluctation [43], Höhn et al. [44] found neither evidence for any of these cellular reactions nor for increase of Ca^{2+} release and protein as well as DNA oxidation while irradiating human dermal fibroblasts at an exposure intensity of 1780 W/m^2 in a temperature-controlled setting. As we were able to create a similar setup involving thermally controlled irradiation, it seems unlikely but not impossible that any of these responses are solely responsible for the inhibitory effect of wIRA on mature chlamydial inclusions. Given the indication that pro-inflammatory cytokines and chemokines are released in the context of wIRA/VIS irradiation on cells alone, and that VIS does not influence mature chlamydial inclusions, it is possible that wIRA overstrains the wide and complex range of anti-apoptotic and immunregulating mechanisms offered by *Chlamydia* [45]. Further studies are needed to prove or disprove this hypothesis.

In addition, while we demonstrate that visible light does not significantly contribute to the anti-chlamydial effect of wIRA/VIS on mature chlamydial inclusions, Wasson et al. [46] has shown an inhibitory effect on chlamydial growth following treatment of infected cells with violet light alone at a wavelength of 405 nm for 88 seconds at 2 and 24 hpi, respectively. Irradiation at early stages of chlamydial infection may undergo a mode of action similar to extracellular EBs and early inclusions might be more vulnerable to visible light, whereas its effect on inclusions in the late stage of chlamydial infection is less pronounced. Also, in the current study, the measured IFU/ml following irradiation of mature inclusions at 40 hpi was equally decreased by wIRA/VIS treatment at all exposure intensities evaluated, while the reduction of EB infectivity following extracellular EB irradiation was irradiation intensity-dependent. Again, these data indicate marked differences for the vulnerability of *Chlamydia* to wIRA/VIS in the late, intracellular stage compared to the extracellular stage of the

developmental cycle, suggesting a complex yet unknown interaction between irradiation, chlamydiae and the host cell.

The effect of wIRA/VIS on extracelluar EBs is in accordance to the Bunsen-Roscoe reciprocity law for ultraviolet (UV) irradiation, which states that “microbial inactivation is dependent on dose and dose is the product of UV intensity expressed in mW per cm^2 and exposure time expressed in seconds” [47]. The effect of treatment duration was not investigated because the exposure time was kept at a constant 20 min and therefore had no impact on our results.

Finally, we wanted to investigate the influence of the infectious dose (MOI) on wIRA/VIS-induced reduction of chlamydial infectivity. We found that the MOI did not affect the relative efficacy of irradiation.

In summary, we, for the first time, show that yet unknown non-thermal effects contribute to the decrease in the infectivity of *Chlamydia* caused by wIRA/VIS irradiation. Additionally, using both animal and human *in vitro* models, we show evidence that the host cell significantly influences the effect of irradiation on intracellular chlamydiae. For example, while wIRA/VIS irradiation of extracelluar EB reduces the subsequent infectivity of these EBs in an irradiation intensity-dependent manner, the detrimental effect on the infectivity of mature chlamydial inclusions occurrs equivalently at all irradiation intensities evaluated. Furthermore, we show that extracellular EBs are highly sensitive to visible light (VIS); whereas, in contrast, we report no significant difference between the reduced infectivity of wIRA/VIS-irradiated or wIRA-irradiated mature chlamydial inclusions. These findings might facilitate the future use of wIRA/VIS as an alternative therapeutic strategy or as a safe and non-harmful method for surface decontamination.

Acknowledgments

The authors would like to thank Carmen Kaiser and Lisbeth Nufer of the laboratory staff at the Institute of Veterinary Pathology, Zurich, for their excellent technical assistance. We thank Werner Müller for instructions using the equipment for wIRA irradiation and immunofluorescence microscopy. We would also like to thank Carolin Rahn for technical assistance, Dr. Cory Ann Leonard for critical review of the manuscript and Prof. Robert V. Schoborg for providing the *C. trachomatis* strain as well as Prof. Johannes Storz for providing the *C. pecorum* strain.

This work was financially supported by the Dr. Erwin Braun Foundation, Basel, Switzerland, awarded to Christian Blenn and Nicole Borel. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. T. Jung, A. Höhn, A.M. Lau, H. Piazena, T. Grune, An experimental setup for the measurement of nonthermal effects during water-filtered infrared A-irradiation of mammalian cell cultures, Photochem. Photobiol. 88 (2012) 371-380. doi: 10.1111/j.1751-1097.2011.01072.x
2. G. Hoffmann, Principles and working mechanisms of water-filtered infrared-A (wIRA) in relation to wound healing, GMS Krankenhhyg. Interdiszip. 2 (2007) Doc54.
3. B.M. Künzli, F. Liebl, P. Nuhn, T. Schuster, H. Friess, M. Hartel, Impact of preoperative local water-filtered infrared A irradiation on postoperative wound healing: a randomized patient- and observer-blinded controlled clinical trial, Ann. Surg. 258 (2013) 887-894. doi: 10.1097/SLA.0000000000000235
4. M. Hartel, G. Hoffmann, M.N. Wente, M.E. Martignoni, M.W. Büchler, H. Friess, Randomized clinical trial of the influence of local water-filtered infrared A irradiation on wound healing after abdominal surgery, Br. J. Surg. 93 (2006) 952-960. doi: 10.1002/bjs.5429
5. C.D. Owens, K. Stoessel, Surgical site infections: epidemiology, microbiology and prevention, J. Hosp. Infect. 70 (2008) 3-10. doi: 10.1016/S0195-6701(08)60017-1
6. H. Marti, M. Koschwanez, T. Pesch, C. Blenn, N. Borel, Water-filtered infrared a irradiation in combination with visible light inhibits acute chlamydial infection, PLoS One 9 (2014) e102239. doi: 10.1371/journal.pone.0102239
7. K. Sachse, P.M. Bavoil, B. Kaltenboeck, R.S. Stephens, C.C. Kuo, R. Rosselló-Móra, M. Horn, Emendation of the family *Chlamydiaceae*: Proposal of a single genus, *Chlamydia*, to include all currently recognized species, Syst. Appl. Microbiol. 38 (2015) 99-103. doi: 10.1016/j.syapm.2014.12.004
8. J.W. Moulder, Interaction of chlamydiae and host cells *in vitro*, Microbiol. Rev. 55 (1991) 143-190.
9. J. Schachter, in: R.S. Stephens (Ed.), *Chlamydia*, American Society of Microbiology, Washington, 1999, pp. 156-157.
10. M. Jelocnik, D. Forshaw, J. Cotter, D. Roberts, P. Timms, A. Polkinghorne, Molecular and pathological insights into *Chlamydia pecorum*-associated sporadic bovine encephalomyelitis (SBE) in Western Australia, BMC Vet. Res. 10 (2014) 121. doi: 10.1186/1746-6148-10-121

11. K.Y. Mohamad, A. Rodolakis, Recent advances in the understanding of *Chlamydophila pecorum* infections, sixteen years after it was named as the fourth species of the *Chlamydiaceae* family, Vet. Res. 41 (2010) 27. doi: 10.1051/vetres/2009075
12. D. Dean, J. Rothschild, A. Ruettger, R.P. Kandel, K. Sachse, Zoonotic *Chlamydiaceae* species associated with trachoma, Nepal, Emerg. Infect. Dis. 19 (2013) 1948-1955. doi: 10.3201/eid1912.130656
13. J. Dugan, D.D. Rockey, L. Jones, A.A. Andersen, Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial inv-like gene, Antimicrob. Agents Chemother. 48 (2004) 3989-3995. doi: 10.1128/AAC.48.10.3989-3995.2004
14. K.M. Sandoz, D.D. Rockey, Antibiotic resistance in chlamydiae, Future Microbiol. 5 (2010) 1427-1442. doi: 10.2217/fmb.10.96
15. R.J. Suchland, K.M. Sandoz, B.M. Jeffrey, W.E. Stamm, D.D. Rockey, Horizontal transfer of tetracycline resistance among *Chlamydia* spp. *in vitro*, Antimicrob. Agents Chemother. 53 (2009) 4604-4611. doi: 10.1128/AAC.00477-09
16. M.R. Hammerschlag, S.A. Kohlhoff, Treatment of chlamydial infections, Expert Opin. Pharmacother. 13 (2012) 545-552. doi: 10.1517/14656566.2012.658776
17. C.A. Leonard, N. Borel, Chronic chlamydial diseases: From atherosclerosis to urogenital infections, Curr. Clin. Micro. Rpt. 1 (2014) 61-72. doi: 10.1007/s40588-014-0005-8
18. R.V. Schoborg, Chlamydia persistence – a tool to dissect chlamydia-host interactions, Microbes Infect. 13 (2011): 649-662. doi: 10.1016/j.micinf.2011.03.004
19. T. Jung, A. Höhn, H. Piazena, T. Grune, Effects of water-filtered infrared A irradiation on human fibroblasts, Free Radic. Biol. Med. 48 (2010) 153-160. doi: 10.1016/j.freeradbiomed.2009
20. N. Borel, C. Dumrese, U. Ziegler, A. Schifferli, C. Kaiser, A. Pospischil, Mixed infection with *Chlamydia* and porcine epidemic diarrhea virus – a new *in vitro* model of chlamydial persistence, BMC Microbiol. 10 (2010) 201. doi: 10.1186/1471-2180-10-201
21. S. Deka, J. Vanover, S. Dessus-Babus, J. Whittimore, M.K. Howett, P.B. Wyrick, R.V. Schoborg, *Chlamydia trachomatis* enters a viable but non-cultivable (persistent) state within herpes simplex virus type 2 (HSV-2) co-infected host cells, Cell. Microbiol. 8 (2006) 149-162. doi:10.1111/j.1462-5822.2005.00608.x

22. D. Dean, in: W. Tasman, E.A. Jaeger (Eds.), Duane's foundations of clinical ophthalmology, Lippincott Williams & Wilkins, Philadelphia, 2010, pp. 678-702.
23. B. Atik, T.T. Thanh, V.Q. Luong, S. Lagree, D. Dean, Impact of annual targeted treatment on infectious trachoma and susceptibility to reinfection, JAMA 296 (2006) 1488-1497. doi: 10.1001/jama.296.12.1488
24. M.J. Burton, Trachoma: an overview, Br. Med. Bull. 84 (2007) 99-116. doi: 10.1093/bmb/ldm034
25. N. Borel, N. Regenscheit, A. Di Francesco, M. Donati, J. Markov, Y. Masserey, A. Pospischil, Selection for tetracycline-resistant *Chlamydia suis* in treated pigs, Vet. Microbiol. 156 (2012) 143-146. doi: 10.1016/j.vetmic.2011.10.011
26. Di Francesco, M. Donati, M. Rossi, S. Pignanelli, A. Shurdhi, R. Baldelli, R. Cevenini, Tetracycline-resistant *Chlamydia suis* isolates in Italy, Vet. Rec. 163 (2008) 251-252. doi: 10.1136/vr.163.8.251
27. K. Schautteet, E. De Clercq, C. Miry, F. Van Groenweghe, P. Delava, I. Kalmar, D. Vanrompay, Tetracycline-resistant *Chlamydia suis* in cases of reproductive failure on Belgian, Cypriote and Israeli pig production farms. J. Med. Microbiol. 62 (2013) 331-334. doi: 10.1099/jmm.0.042861-0
28. B.M. Jeffrey, R.J. Suchland, S.G. Eriksen, K.M. Sandoz, D.D. Rockey, Genomic and phenotypic characterization of *in vitro*-generated *Chlamydia trachomatis* recombinants, BMC Microbiol. 13 (2013) 142. doi: 10.1186/1471-2180-13-142
29. J. Cadet, E. Sage, T. Douki, Ultraviolet radiation-mediated damage to cellular DNA. Mutat. Res. 571 (2005) 3-17. doi: 10.1016/j.mrfmmm.2004.09.012
30. G.J. Fisher, H.S. Talwar, J. Lin, P. Lin, F. McPhillips, Z. Wang, X. Li, Y. Wan, S. Kang, J.J. Voorhees, Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin *in vivo*, J. Clin. Invest. 101 (1998) 1432-1440. doi: 10.1172/JCI2153
31. C. Jhappan, F.P. Noonan, G. Merlino, Ultraviolet radiation and cutaneous malignant melanoma, Oncogene 22 (2003) 3099–3112. doi:10.1038/sj.onc.1206450
32. J.G. Peak, M.J. Peak, Comparison of initial yields of DNA-to-protein crosslinks and single-strand breaks induced in cultured human cells by far- and near-ultraviolet light, blue light and X-rays, Mutat. Res. 246 (1991) 187-91. doi:10.1016/0027-5107(91)90121-4

33. J.L. Ravanat, T. Douki, J. Cadet, Direct and indirect effects of UV radiation on DNA and its components, J. Photochem. Photobiol. B 63 (2001) 88-102. doi:10.1016/S1011-1344(01)00206-8
34. S. Schieke, H. Stege, V. Kürten, S. Grether-Beck, H. Sies, J. Krutmann, Infrared-A radiation-induced matrix metalloproteinase 1 expression is mediated through extracellular signal-regulated kinase 1/2 activation in human dermal fibroblasts, J. Invest. Dermatol. 119 (2002) 1323-1329. doi:10.1046/j.1523-1747.2002.19630.x
35. V.H. Hu, M.J. Holland, M.J. Burton, Trachoma: protective and pathogenic ocular immune responses to *Chlamydia trachomatis*, PLoS Negl. Trop. Dis. 7 (2013) e2020. doi: 10.1371/journal.pntd.0002020
36. J.A. Byrne, P.A. Fernandez-Ibañez, P.S.M. Dunlop, D.M.A. Alrousan, J.W.J. Hamilton, Photocatalytic Enhancement for solar disinfection of water: a review, Int. J. Photoenergy 2011 (2011) 12 pages. http://dx.doi.org/10.1155/2011/798051
37. K.G. McGuigan, R.M. Conroy, H.J. Mosler, M. du Preez, E. Ubomba-Jaswa, P. Fernandez-Ibañez, Solar water disinfection (SODIS): a review from bench-top to roof-top, J. Hazard Mater. 235-236 (2012) 29-46. doi: 10.1016/j.jhazmat.2012.07.053
38. M. Wegelin, S. Canonica, K. Mechsner, T. Fleischmann, F. Pesaro, A. Metzler, Solar water disinfection: scope of the process and analysis of radiation experiments, J. Water SRT-Aqua. 43 (1994) 154-169.
39. R. Reinhold, K. Sachse, B. Kaltenboeck, *Chlamydiaceae* in cattle: commensals, trigger organisms, or pathogens?, Vet. J. 189 (2011) 257-267. doi: 10.1016/j.tvjl.2010.09.003
40. D. Longbottom, L.J. Coulter, Animal chlamydioses and zoonotic implications. J. Comp. Pathol 128 (2003) 217-244. doi: 10.1053/jcpa.2002.0629
41. J.C. Nietfeld, Chlamydial infections in small ruminants, Vet. Clin. North Am. Food Anim. Pract. 17 (2001) 301-314.
42. O.S. Harb, L.Y. Gao, Y. Abu Kwaik, From protozoa to mammalian cells: a new paradigm in the life cycle of intracellular bacterial pathogens, Environ. Microbiol. 2 (2000) 251-265. doi: 10.1046/j.1462-2920.2000.00112.x
43. P. Schroeder, C. Pohl, C. Calles, C. Marks, S. Wild, J. Krutmann, Cellular response to infrared radiation involves retrograde mitochondrial signaling, Free Radic. Biol. Med. 43 (2007) 128-135. doi: 10.1016/j.freeradbiomed.2007.04.002

44. A. Höhn, P. Hartmann, V. Gebhart, J. Sonntag, T. Grune, T. Jung, Actual isothermal effects of water-filtered infrared A-irradiation, Photochem. Photobiol. 91 (2015) 887-894. doi: 10.1111/php.12439. Epub 2015 Mar 12.
45. R.J. Bastidas, C.A. Elwell, J.N. Engel, R.H. Valdivia, Chlamydial intracellular survival strategies, Cold Spring Harb. Perspect. Med. 3 (2013) a010256. doi: 10.1101/cshperspect.a010256.
46. C.J. Wasson, J.L. Zourelias, N.A. Aardsma, J.T. Eells, M.T. Ganger, J.M. Schober, T.A. Skwor, Inhibitory effects of 405 nm irradiation on *Chlamydia trachomatis* growth and characterization of the ensuing inflammatory response in HeLa cells, BMC Microbiol. 12 (2012) 176. doi: 10.1186/1471-2180-12-176
47. T.D. Cutler, J.J. Zimmerman, Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents, Anim. Health Res. Rev. 12 (2011) 15-23. doi: 10.1017/S1466252311000016

Supporting Information

Figure S1.

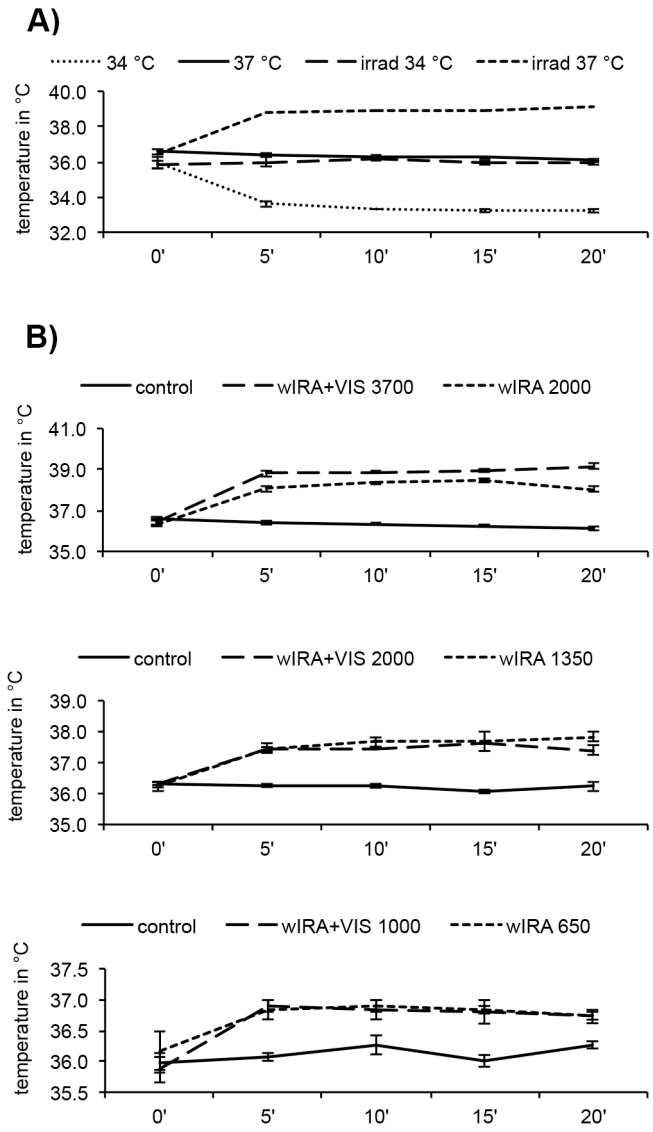


Figure S1.

Additional temperature profiles.

Figure S2. *C. trachomatis* in HeLa

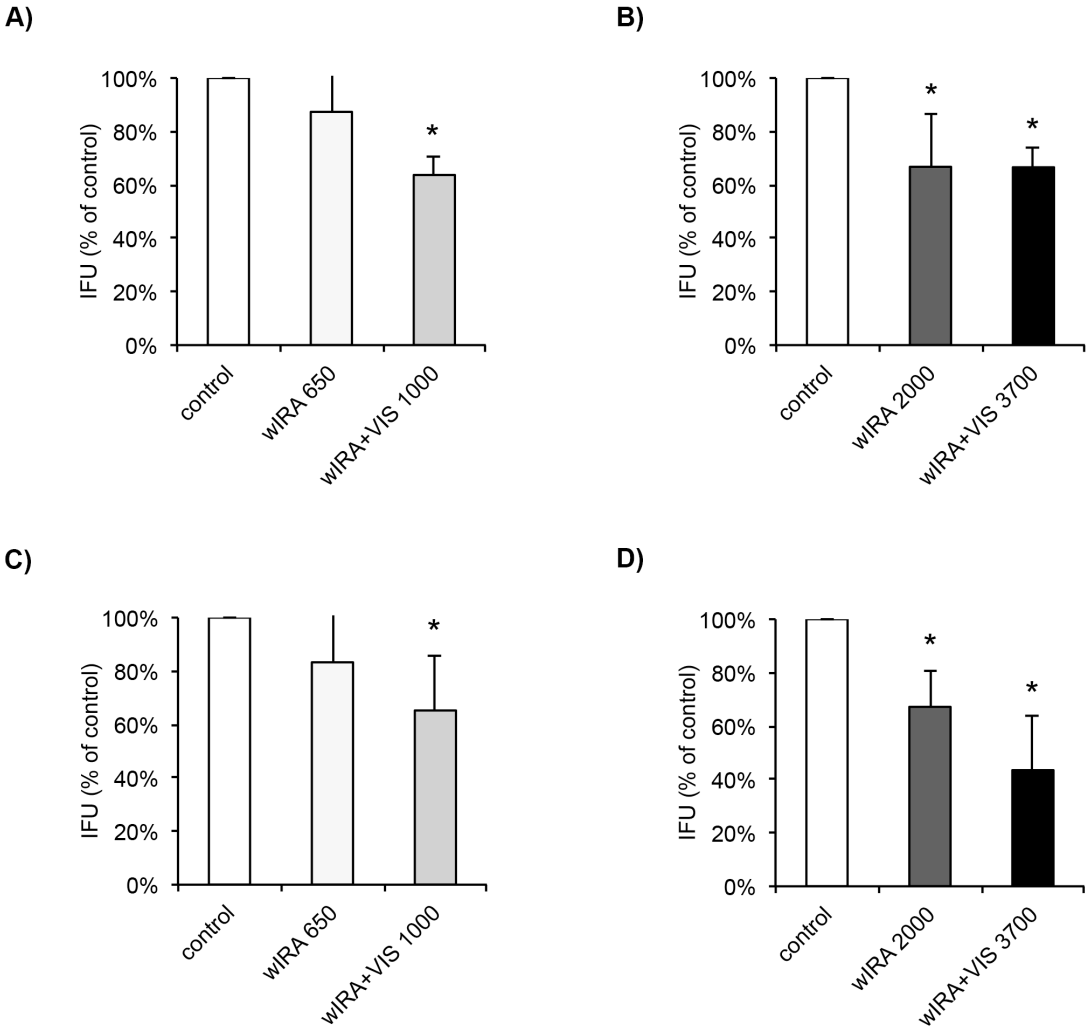


Figure S2.

The effect of wIRA/VIS and wIRA alone on mature *C. trachomatis* inclusions at MOI 1 (Fig. S2A, B) and MOI 0.01 (Figure S2C, D).

Figure S3. *C. pecorum* in HeLa

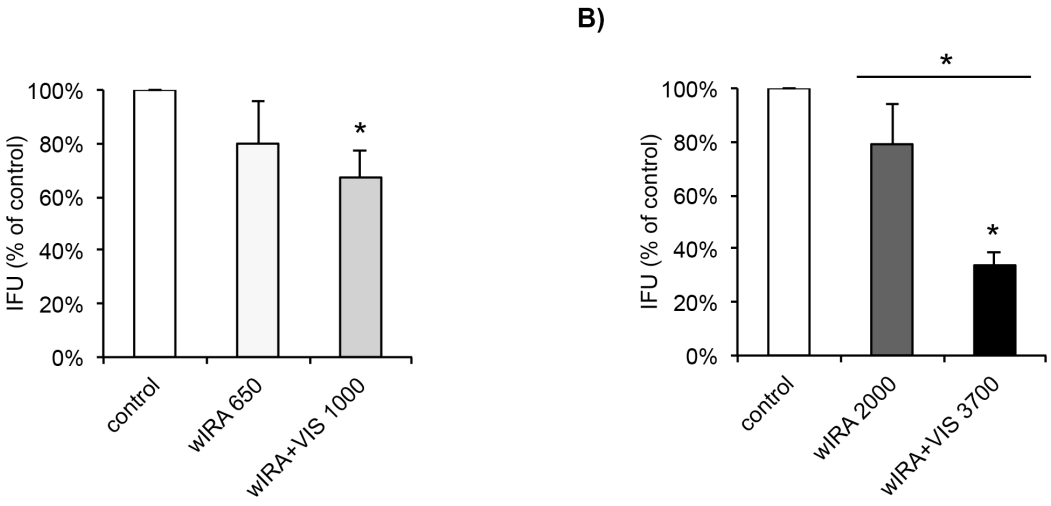


Figure S3.

The effect of wIRA/VIS and wIRA alone on *C. pecorum* EBs at MOI 0.1 prior to infection of HeLa cells at A) low (1000 W/m^2) and B) high (3700 W/m^2) exposure intensities.